REMARKS

With this response, claims 1-19, and 34-36 are pending in this application. Claims 1, 5, 10, 14, 18, and 34 have been amended. Support for the amendments to claims 1, 5, 10, and 18 can be found in the specification at p. 11, lines 29-31. Support for the amendments to claim 14 can be found in the specification at p. 17, lines 6-10. Support for the amendments to claim 34 can be found in the specification at p. 2, line 9-10 and at p. 11, lines 29-31.

Claims 41-45 are newly added. Support for these claims can be found in the specification at p. 11, lines 29-31.

No new matter was added by the amendments or new claims.

I. 35 U.S.C. §112, Second Paragraph Rejection

A. Claims 1-19

Reconsideration is requested of the rejection of claims 1-19 under 35 U.S.C. 112, second paragraph as being indefinite for reciting the phrase "detecting the presence of said dye" as the last step in a method of labeling cells. The Office asserts that there is insufficient antecedent basis for this limitation.

The phrase "detecting the presence of said dye" has been deleted from claims 1, 5, 10, and 18, thereby obviating the rejection of claims 1-19 under 35 U.S.C. 112, second paragraph based upon the presence of the phrase.

B. Claims 34-36

Reconsideration is requested of the rejection of claims 34-37 under 35 U.S.C. 112, second paragraph as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. Citation is made to MPEP 2172.01. Applicants interpret this rejection to be a rejection for failing to point out and distinctly claim the invention and will treat the rejection as such. Additionally, while the Office has rejected claims 34-37, claim 37 was withdrawn from

consideration by the Office as being drawn to a non-elected group. Applicants assume the Office meant instead to reject claims 34-36 and will treat the rejection as such.

The phrase "such that expression of the proteins occurs" has been added to claim 34, thereby obviating the rejection of claims 34-36 under 35 U.S.C. 112, second paragraph. Support for this language can be found in the specification, p. 2, line 9-10, wherein it is noted that the expression of GFP in cells results in fluorescence.

II. 35 U.S.C. §102(b) Rejection

A. Haugland et al. or Yue

Reconsideration is requested of the rejection of claims 1-3 and 17 under 35 U.S.C. 102(b) as being unpatentable over Haugland et al. (U.S. Patent No. 5,436,134) or Yue (U.S. Patent No. 5,656,449).

As amended, claims 1 and 17 (via the amendment of dependent claim 10, which serves as the basis of rejected dependent claim 17) are directed to methods of labeling individual cells by propelling dye-coated metal particles into contact with target cells, such that the contact is for a time sufficient to cause labeling of the target cells by release of the dye from the metal particles.

Haugland et al ("Haugland") discloses novel dyes and the use of those dyes to stain a sample containing a nucleic acid, incubating the sample for a time sufficient to obtain a detectable flourescent response, and observing the flourescent response. While Haugland et al. states that suitable means for transporting the dyes across cell membranes includes "bombardment with solid particles coated with or in the presence of the dyes," Column 7, lines 59-60, none of the examples demonstrate the use of bombardment. Furthermore, Haugland et al. does not disclose the use of propulsion or bombardment with metal particles to label cells or the use of propulsion or bombardment to label anything other than nucleic acids.

Yue discloses novel dyes and the use of those dyes to stain a sample containing a nucleic acid, incubating the sample for a time sufficient to obtain a detectable flourescent response, and observing the flourescent response. While Yue states that suitable means for transporting the

dyes across cell membranes includes "bombardment with solid particles coated with or in the presence of the dyes," Column 9, lines 64-65, none of the examples demonstrate the use of bombardment. Yue does not disclose the use of propulsion or bombardment with metal particles to label cells or the use of bombardment to label anything other than nucleic acids.

Unlike Haugland and Yue, claims 1-3 and 17 are not methods of labeling nucleic acids, but are methods of labeling cells. Therefore, unlike Haugland and Yue, the dye would not need to enter the cell nucleus. Specifically, it is noted in the present application that the particles need not come in contact with the cell nucleus[1] and that generally the labeling of the target cell is the labeling of the cell membrane.[2] Furthermore, Haugland and Yue do not disclose the use of propulsion or bombardment with a dye-coated metal particle. The only references to bombardment are the brief phrases cited above, and these statements clearly do not suggest the use of a metal particle. Therefore, Haugland and Yue fail to disclose each and every element of claims 1 and 17, and accordingly fail to anticipate the claimed invention

Claims 2 and 3 depend from claim 1 and are patentable over Haugland and Yue for the reasons stated with respect to claim 1 and by reason of the additional requirements which they introduce.

B. Fitzpatrick-McElligott

Reconsideration is requested of the rejection of claims 1-2, 4, 10, 11, 13, and 17 under 35 U.S.C. 102(b) as being unpatentable over Fitzpatrick-McElligott (U.S. Patent No. 5,466,587).

Fitzpatrick-McElligott discloses the use of particles having substantially pure carbonaceous surfaces to administer to cells a biological substance via particle bombardment. Examples of biological substances includes biological stains. Fitzpatrick-McElligott does not

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[1] Specification, p. 10, lines 8-9.

[2] Specification, p. 10, lines 16-19.

disclose the use of propulsion or bombardment to label cells with any type of particle other than one that has a substantially pure carbonaceous surface.

Unlike Fitzpatrick-McElligott, independent claims 1 and 10 are not methods of labeling cells using particles having substantially pure carbonaceous surfaces, but are instead methods of labeling cells using metal particles. Therefore, Fitzpatrick-McElligott fails to disclose each and every element of claims 1 and 10.

Claims 2 and 4 depend from claim 1, and claims 11, 13, and 17 depend from claim 10. These dependent claims are patentable over Fitzpatrick-McElligott for the reasons stated with respect to claims 1 and 10 and by reason of the additional requirements which they introduce.

III. 35 U.S.C. §~~102(b)~~ 103 Rejection

A. Haugland, Yue, and Fitzpatrick-McElligott in view of Magrassi et al., Gan et al., and Gee et al., and further in view of Pichersky

Reconsideration is requested of the rejection of claims 1-19 under 35 U.S.C. 103 as being unpatentable over Haugland, Yue, and Fitzpatrick-McElligott in view of Magrassi et al. (1987), Gan et al. (1999), Gee et al. (U.S. Patent No. 5,888,829), and further in view of Pichersky (U.S. Patent No. 5,436,134).

As stated in greater detail above in Section I.A., claims 1 and 10, as amended, are directed to methods of labeling individual cells by propelling dye-coated metal particles into contact with the target cells.

Haugland and Yue disclose methods of labeling nucleic acids using dyes. Neither disclose the labeling of cells or the use of dye-coated metal particles.

Fitzpatrick-McElligott discloses the use of particles having substantially pure carbonaceous surfaces to administer to cells a biological substance via particle bombardment. It does not disclose the use of dye-coated metal particles.

Magrassi et al. ("Magrassi") discloses cationic mitochondrial dyes used to stain living nerve terminals. Contrary to the Office's assertions, these dyes are not lipophilic. Magrassi does

yes they are
- see evidence

not disclose labeling cells with a lipophilic dye or labeling cells by propulsion of lipophilic dye-coated metal particles.

Gan et al. ("Gan") discloses the labeling of individual axon terminal by iontophoretic application of lipophilic dyes such as DiO and DiI. Gan does not disclose labeling cells by propulsion of lipophilic dye-coated metal particles.

Gee et al. ("Gee") discloses the use of the Ca^{2+} ion indicator Oregon Green® BAPTA. Gee does not disclose labeling cells by propulsion of dye-coated metal particles.

Pichersky discloses the delivery of DNA coated particles to a cell by acceleration, such as the Biolistics Particle Delivery System. Pichersky further states that the particles can be accelerated through a stainless steel or Nytex screen. It is believed that the screen reduces the size of aggregate particles and leads to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are to large. Pichersky does not disclose the delivery of dye to cells, the use of dye-coated metal particles to deliver the dye, or the use of the screen to control the distribution of the dye particles and improve imaging of the dyed cells.

Dellaporta is cited as having the same disclosure, but is not relied upon as duplicative. Furthermore, Dellaporta notes that DNA segments can be delivered to plant cells via microprojectile bombardment, wherein "exemplary particles include those comprised of tungsten, gold, platinum, and the like."[3] Dellaporta does not disclose the labeling of cells by propulsion with dye-coated particles.

The combination of the references fails to make the claimed invention obvious as there is a lack of motivation to combine the references. Specifically, Yue and Haugland, while mentioning bombardment, fail to specifically demonstrate bombardment and fail to disclose a metal particle. These references, therefore, fail to provide any guidance as to a how to perform bombardment or of a particular type of particle.

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[3] Column 6, lines 35-44.

Fitzpatrick-McElligott specifically **requires** that the particle used to deliver the dye have a substantially pure carbonaceous surface. This would lead one to believe that there is something unique to a particle with a substantially pure carbonaceous surface that allows dyes to adhere to the particle, and that only particles with a substantially pure carbonaceous surface can be used in conjunction with dyes.

Dellaporta articulates that biolistic particle delivery can be used to deliver DNA to cells using exemplary particles such as tungsten, gold, or platinum. However, this reference is limited to the delivery of DNA only, making no suggestion as to the delivery of other materials such as dyes. This would lead one to believe that there is something unique to metal particles that specifically allows nucleic acids to adhere to such particles or that specifically allows such particles to be used as described.

Therefore, there is a lack of motivation within the references themselves to combine the references such that one would use bombardment to deliver metal particles coated with dyes. Absent this lack of motivation, the Office has failed to establish a *prima facie* case of obviousness as to dependent claims 1, 5, and 18.[4]

Furthermore, with respect to the use of a plurality of dye-coated particles and a macroprojectile stopping means, while Pichersky and Dellaporta demonstrate the use of a microprojectile screen to reduces the size of aggregate particles, they do so in an effort to obtain a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large.[5] They do not disclose the use of the microprojectile screen to prevent the clustering of particles onto target cells or tissues in an effort to control the distribution of the dye particles and improve imaging of the dyed cells. Such a limitation is not taught in Pichersky or Dellaporta, nor is it cured by the combination of these references with any of the additionally cited references. Therefore, because the combination of references fails to

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[4] MPEP §2142.

[5] Pichersky, column 5, lines 49-54; Dellaporta, column 6, line 64 t0 column 7, line 2.

teach or suggest all such claim limitations, the Office has failed to establish a *prima facie* case of obviousness with respect to claim 14 or 15.[6]

Claims 2-4 depend from claim 1, claims 6-9 depend from claim 5, claims 11-17 depend from claim 10, and claim 19 depends from claim 18. These dependent claims are patentable over Haugland, Yue, and Fitzpatrick-McElligott in view of Magrassi et al., Gan et al., and Gee et al., and further in view of Pichersky and Dellaporta for the reasons stated with respect to claims 1, 5, 10, 15, and 18 and by reason of the additional requirements which they introduce.

B. Wong et al. in view of Tsien and Matz

Reconsideration is requested of the rejection of claims 34-36 under 35 U.S.C. 103(a) as being unpatentable over Wong et al. (1998) in view of Tsien (1998) and Matz (1999). Although the Office rejected claims 34-38, claims 37 and 38 were withdrawn from consideration by the Office as being drawn to a non-elected group. Applicants assume the Office meant instead to reject claims 34-36 and will treat the rejection as such.

Claim 34, as amended, teaches a method of labeling individual cells comprising propelling a plurality of metal particles containing a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra toward a plurality of cells to cause the particles to enter into and reside in the cells such that expression of the proteins occurs.

Wong et al. ("Wong") discloses staining retinal ganglion cells with green fluorescent protein ("GFP") by biolistic methods. Wong does not disclose the use of metal particles, the use of a plurality of metal particles, or the use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra on metal particles.

Tsien discloses that there are many different GFPs having different fluorescent properties. Tsien does not disclose bombardment, the use of metal particles, the use of a plurality of metal

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[6] MPEP §2142.

particles, or the use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra on metal particles.

Matz et al ("Matz") discloses that there are many flourescent proteins that are analogous to GFP and that can be used for the same purpose. Matz does not disclose bombardment, the use of metal particles, the use of a plurality of metal particles, or the use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra on metal particles.

Even when combined, these three references fail to teach or suggest all the claim limitations of the rejected claims, as the references do not disclose the use of metal particles in bombardment, the use of a plurality of metal particles in bombardment, or the use of a plurality of nucleic acid sequences encoding fluorescent proteins having different emission spectra on metal particles in bombardment. Because the combination of references fails to teach or suggest all such claim limitations, the Office has failed to establish a *prima facie* case of obviousness with respect to claim 34.[7]

Claims 35 and 36 depend from claim 34, and are patentable over Wong in view of Tsien and Matz for the reasons stated with respect to claim 34 and by reason of the additional requirements which they introduce.

IV. Additional Art

The Examiner listed two additional pieces of art in the Notice of References (U.S. Patent 6,290,991 and WO 01/69244). Neither is cited within the Office action. Furthermore, WO 01/69244 is the PCT filing corresponding to the present application, having the same filing date (March 9, 2001) and claiming priority to the same provisional application (U.S. Application Serial No. 60/188,370, filed March 10, 2000) as the present application.

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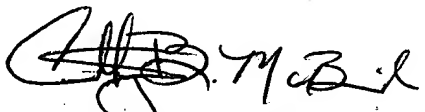
[7] MPEP §2142.

CONCLUSION

In light of the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-19 and 34-36 under 35 U.S.C. 112, second paragraph, of claims 1-4, 10, 11, 13, and 17 under 35 U.S.C. 102(b), and of claims 1-19 and 34-36 under 35 U.S.C. 103(a).

Applicants request an extension of time to and including December 6, 2002, for filing a response to the above-mentioned Office action. The Commissioner is hereby authorized to charge the applicable extension fee to Deposit Account No. 19-1345.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (amended) A method for labeling of individual cells comprising:

providing at least one target cell;

providing at least one **metal** particle coated with at least one dye;

propelling said coated particle toward said target cell to thereby cause said coated particle to contact said cell for a time sufficient to cause labeling of said target cell by release of said dye from said particle**[; and**

detecting the presence of said dye].

5. (amended) A method for labeling of individual cells comprising:

providing at least one target cell, said target cell having a cell membrane;

providing at least one **metal** particle coated with at least one lipophilic dye;

propelling said coated particle toward said target cell to thereby cause said coated particle to contact said cell membrane for a time sufficient to cause labeling of said target cell by release of said dye from said particle**[; and**

detecting the presence of said dye].

10. (amended) A method for labeling of individual cells comprising:

providing a plurality of target cells;

providing a plurality of **metal** particles coated with at least one dye;

propelling said coated particles toward said target cells to thereby cause said coated particles to contact said cells for a time sufficient to cause labeling of said target cells by release of said dye from said particles**[; and**

detecting the presence of said dye].

14. (amended) The method of claim 13, further comprising **controlling distribution of the dye coated metal particles to improve imaging of the target cells by** causing said at least one macroprojectile to contact a macroprojectile stopping means before contacting said target cells, said macroprojectile stopping means capable of stopping said macroprojectile while allowing at least one coated particle to continue toward said target cells.

18. (amended) A method for labeling of individual cells comprising:

providing at least one **metal** particle containing at least one lipophilic dye selected from the group consisting of DiO, DiI, DiD and any combination thereof to form a coated particle;

providing at least one target cell, said target cell having a cell membrane;

propelling said coated particle toward said target cell to thereby cause said coated particle to contact said cell membrane for a time sufficient to cause labeling of said target cell by release of said dye from said particle**[; and**

detecting the presence of said dye].

34. (amended) A method for labeling individual cells comprising:

providing a plurality of target cells;

providing a plurality of **metal** particles containing a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra; and

propelling said plurality of particles toward said plurality of cells to cause said particles to enter said cells and reside in said cells **such that expression of the proteins occurs**.

Claims 41-45 are newly added.